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Research advance and superiority of microdiesel production with biowastes

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Lignocellulosic biowastes can be utilized to produce microdiesel, a promising alternative energy source for limited crude oil and food based biofuels. As an alternative fuel, microdiesel produced from biowastes it has superior environmental benefits, economically competitive, producible in sufficient quantities and the energy conversion efficiency is also estimated in the article in theory. Microdiesel derived from biowastes is a potential renewable and carbon neutral alternative to petroleum fuels. Unfortunately, microdiesel from biowastes cannot realistically satisfy even a small fraction of the existing demand for transport fuels. As demonstrated here, microdiesel appears to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels. Approaches for making microdiesel economically competitive with petrodiesel are also discussed.

Key words: Microdiesel, lignocellulosic biowastes, environmental benefits, potential supply.

INTRODUCTION

Energy security and climate change imperatives require large-scale substitution of petroleum based fuels as well as improved vehicle efficiency (Farrell et al., 2006; Lund, 2007). Over the past three decades, the desires to establish national energy self-reliance and to develop alternatives for the finite fossil fuel resources have resulted in the development of fuel technologies that are based on the use of renewable agricultural-based materials as feedstock.

Among the diverse range of promising alternatives, PHB (Poly -hydroxybutyric acid), bioethanol and biodiesel have become the hot spot of research. PHB (Figure 1) has properties similar to petroleum derived synthetic plastics like polypropylene (PP) and is completely biodegradable in environment (Bucci et al., 2007; Khardenavis et al., 2006; Yang et al., 2006). Bioethanol and biodiesel are viable biofuel as biodegradable, renewable and non-toxic fuel. Bioethanol is fermented from sugars, starches or cellulosic biomass (Borzani, 2006; Sun et al., 2002). Figure 2 shows the flow chart for the production of bioethanol from biomass (Demirbas, 2007; Qu et al., 2006). Compared with bioethanol, biodiesel has shown to give engine performance generally comparable to that of conventional diesel fuel while reducing engine emissions of particulates, hydrocarbons and carbon monoxide (Hassan et al., 1993; Swanson et al., 2007). Biodiesel is generally prepared by trans-esterification of plant with methanol as described in Figure 3 (Mustafa, 2005; Ozturk et al., 2006; Tao et al., 2006).

Microdiesel is biodiesel from oils of various microorganisms, which is now a potential feedstock for biodiesel production due to their specific characteristics such as they are not affected neither by seasons nor by climates, they have high lipid content, can be produced from a wide variety of sources with short period of production, especially from agricultural and forestry residues with abundant supply and so on (Xue et al., 2006; Zhao, 2005a). If biodiesel could be produced from low-input biomass grown on agricultural marginal land or from waste biomass, it could provide much greater supplies and environmental benefits than food-based biofuels (Hill, 2006; Kalscheuer, 2006a). There are mainly three processes involved in the conversion: hydrolysis of cellulose and hemicellulose to produce reducing sugars, conversion of the reducing sugars to microbial oil and further trans-esterification to microdiesel (Figure 4).

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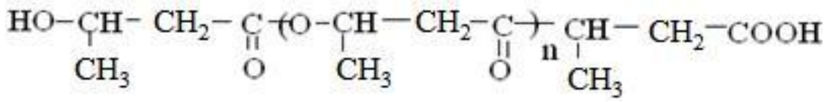

Figure 1. The structure of PHB (Yang et al., 2006).

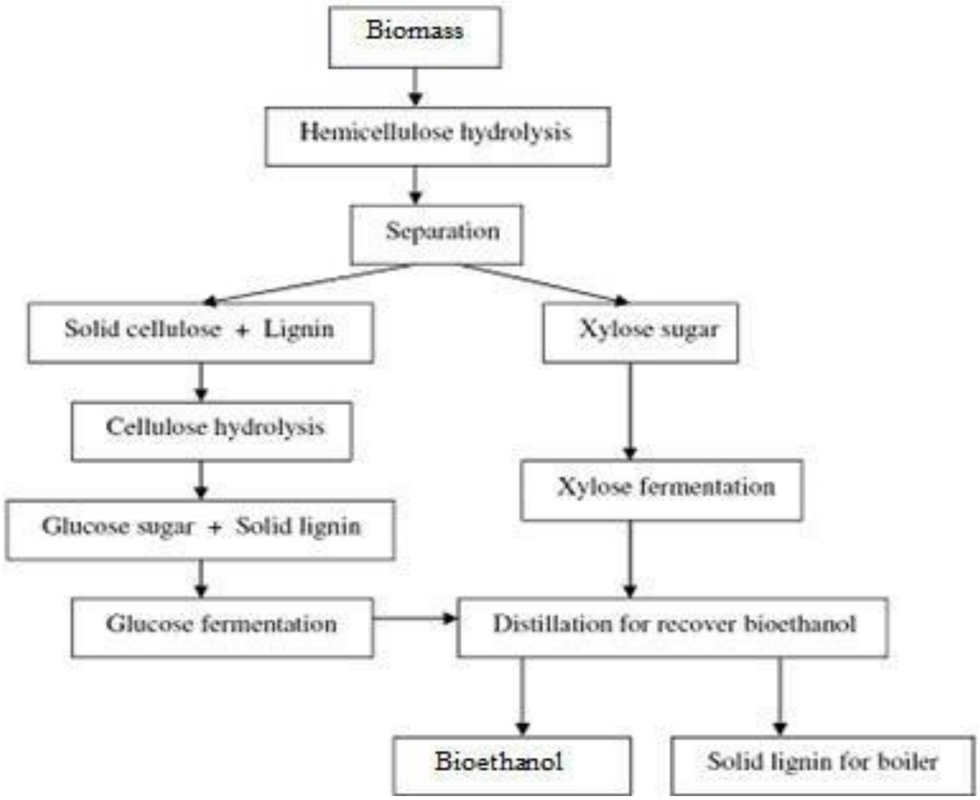

Figure 2. Flow chart for the production of bioethanol from biomass (Demirbas, 2007).

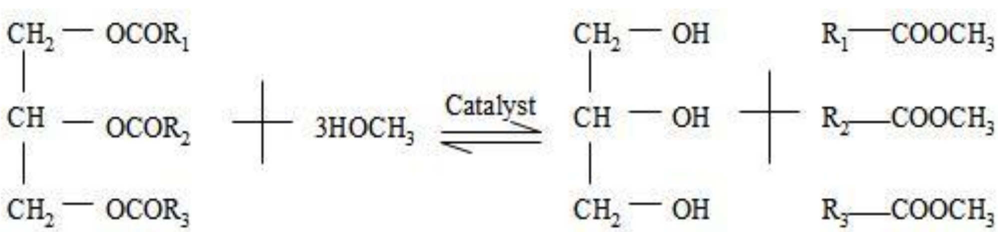

Figure 3. Trans-esterification of oil to biodiesel. R1-3 are hydrocarbon groups (Chisti Yusuf, 2007).

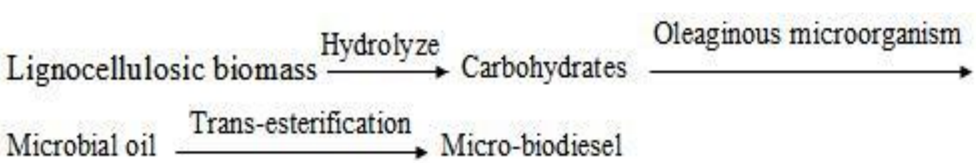

Figure 4. The process of lignocellulosic materials to biodiesel (Tao et al., 2006).

Table 1. The composition of some common agricultural and forestry residues.

Source	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)
Corn stover	38 - 40	28	7 - 21	3.6 - 7.0
Coir	36 - 43	0.15 - 0.25	41 - 45	2.7 - 10.2
Bagasse	32 - 48	19 - 24	23 - 32	1.5 - 5
Wheat straw	33 - 38	26 - 32	17 - 19	6 - 8
Rice straw	28 - 36	23 - 28	12 - 14	14 - 20
Sorghum stalks	27	25	11	- - -
Barley straw	31 - 35	27 - 38	14 - 19	2 - 7
Leaves	15 - 20	80 - 85	0	- - -

Moreover, unlike oil crops, microorganisms appear to be the only source of biodiesel that has the potential to completely displace fossil diesel. Microorganisms grow extremely rapidly and many are exceedingly rich in oil.

The potential microdiesel yield from biowastes

Shifiting society's dependence away from petroleum to renewable biomass resources is generally viewed as an important contributor to the development of a sustainable industrial and effective management of greenhouse gas emission (Arthur, 2006). Biofuels would provide greater benefits only if the biomass feed stocks were producible with low agricultural input (less fertilizer, pesticide and energy) and were producible on land with agricultural value, however, food-based biofuels could not do particularly well on the criteria as corn requires large N, P and pesticide input, most crops require fertile land. Moreover, food-based biofuels can meet but a small portion of transportation energy needs.

On the other hand, there is a huge amount of low value or waste lignocellulosics materials that are currently burned or wasted. Utilization of lignocellulosics could meet the criteria well and has the potential to significantly reduce the price of biodiesel compared with food based biofuels. Biomass such as agricultural crops and residues, forest resources and residues, municipal wastes is the largest source of lignocellulosics in the world. Approximately 2×10^{11} tons of lignocellulosics are produced every year and organic agricultural and forestry by-products are annually renewable, available in abundance and of limited value at present (Chen et al., 2006; Reddy et al., 2005). By-products produced from the cultivation of corn, wheat, rice, sorghum, barley, sugarcane and coconuts are the major sources of agro-based lignocellulosics which contains high content of cellulose and hemicellulose as described in Table 1 (Reddy et al., 2005; Sun et al., 2002). However, the biomass resource is usually wasted and burnt, which results in environmental pollution, caused by global addition of carbon dioxide, a gas contribution to the greenhouse effect. At the same time, the cellulose and hemicellulose could provide sugar for bioconversion to microbial oil by oleaginous microbes, which can be further transferred to biodiesel by trans-esterification.

By microbes, biomass, especially lignocellulosic biomass could be converted to oil which could be further converted to biodiesel. The biodiesel made from the process has the potential to be much cheaper than biodiesel made from vegetable oils. According to the Figure 4, it is estimated that the energy conversion efficiency of corn stover to biodiesel and differences in calorific value between various biomass resources are taken into account for the purpose of calculation. The corn stover contains cellulose (40%), hemicellulose (25%), lignin (17%), its quantity of heat is about 17.8×10^3 kJ/kg, because oleaginous microorganism can use glucose and pentose (xylose and pectinose). In theory, corn straw can produce micro-biodiesel 233 kg and glycerol 22.8 kg per ton (fatty acid calculates as stearic acid). As a result, the energy conversion efficiency is about 55% (Kay, 2006; Tao et al., 2006; Zhao et al., 2005b). In the paper, we suppose the lignocellulosics as corn stalk for granted, 2×10^{11} tons of lignocellulosics would mean 4.66×10^{10} tons of microdiesel and 4.56×10^9 tons of glycerol. Such a large amount of biofuel would greatly ease or even solve the energy crisis worldwide completely.

Environmental benefits of microdiesel from biowastes

Biofuel from crops has become attractive recently because of its environmental benefits and the fact that it is made from renewable resources. Comparing with petrol and its derivatives, using biofuel as an alternative fuel has a lot of environmental advantages such as almost zero emissions of sulfates and a small net contribution of CO_2. However, both corn and soybean production for bioethanol and biodiesel have negative environmental impacts through movement of agrochemicals, especially nitrogen (N), phosphorus (P) and pesticides from farms to other habitats and aquifers. Agricultural N and P are transported by leaching and surface flow to surface, ground and coastal waste causing eutrophication, loss of biodiversity and elevated nitrate and nitrite in drinking wells (Hill, 2006; Mclaughlin et al., 1997).

Agro-based lignocellulosics are potential renewable energy resources and current disposal methods for these agricultural residues have caused widespread environmental concerns, for example, disposal of rice and wheat straw by open -field burning has caused air pollution. As microdiesel is usually produced from biowastes such as byproducts from cultivation of corn, wheat, rice, sorghum, barley, sugarcane and coconuts, the process doesn't impose threat to the environment as the production of these biowastes does not need agrochemical and pesticides (Hoffert et al., 2002). Moreover, the cells after lipid extraction have high content protein and other nutrients, which would be a promising source of organic fertilizer and boost the development of green agriculture (Jiang et al., 2006; Lal et al., 2007). Utilization of biowastes as energy with high efficiency and rationality not only meets the demands for energy, but also provides a basis for environmental protection and sustainable development of the society (Pimentel, 2006; Zeng et al., 2007).

Economic competitiveness and net social benefits of microdiesel from biowastes

Fossil energy use imposes environmental costs not captured in market prices, whether a microdiesel provides net benefits to society depends not only on whether it is cost competitive, but also on its environmental costs and benefits, when it is the fossil fuel alternatives (Schneider et al., 2003) . Subsidies for otherwise economically uncompetitive biofuels are justified if their life- cycle environmental impacts are sufficiently less for alternatives.

However, biodiesel is currently more expensive than conventional diesel due to high cost share of 70 - 85% for raw material. If produced from waste biomass, could provide much greater supplies and environmental benefits than food based biodiesel and greatly lower the price to be more competitive (Schubert, 2006).

Further increases in petroleum prices improves the cost competitiveness for biofuels and we are disappointed that the prices of crops increase rapidly in China too, which will inevitably increase the prices of biofuels from crops. As the biowastes are rather cheap, even without cost, the microdiesel produced from biowaste is sure to be much cheaper and economically competitive. The cost of microdiesel production from lignocellulosic materials now is relatively high based on current technologies and the main challenges are the low yield and high cost of the hydrolysis process. Microdiesel from lignocellulosics production will be profitable because of large subsidies even when not cost competitiveness because of technological problems in utilizing lignocellulosics. The government should lay strong foundation and infrastructures for supporting and promoting the use of renewable energy and energy conservation especially in the form of necessary legislation and support funds, as this technology turns ordinary low-value materials such as corn stalks, sawdust, or organic waste into fuel.The technology not quite lead into gold, but maybe more valuable for China, for cutting pollution and reducing dependence on foreign oil or others forms of fuel (Tao et al., 2006).

Agricultural green energy production will be the principal contributor in economic development of China as most of people live in rural areas. It is believed that integrated community development contributes to push up socio-economic development of the country.

Research advance of microdiesel

The globally escalating demands for both food and energy have raised concerns about the potential for food based biofuels to be sustainable, abundant and environmentally beneficial energy sources. Current biofuel production competes for fertile land with food production, increases pollution from fertilizers and pesticides and threatens biodiversity when natural lands are converted to biofuel production (Tilman et al., 2006). On the other hand, there is no advanced biofuels industry today and the biofuel supply chain requires many participants working closely together (Gutterson et al., 2009). A way of addressing this sensitive issue could be through the bio-development of biowaste as an alternative and renewable energy source (VanWyk, 2001).

Unlike other oil crops, microbes grow extremely rapidly and many are exceedingly rich in oil. Oil content of 20 - 50% are quite common while some microorganisms could exceed 70% by weight of dry biomass such as *Cryptococcus albidus*, *Cryptococcus albidun*, *Lipomyces*, *Trichospiron pullulans*, *Lipomy slipofer*, *Rhodotorula glutinis* and *Rhodosporidium toruloides* (oleaginous yeast); *Asoergullus terreus*, *Claviceps purpurea*, *Tolyposporium*, *Mortierella alpina* and *Mortierlla isabellina* (oleaginous fungus); *diatom* and *Spirulina* (oleaginous alga) (Rattry, 1984).

Peng et al. (2007) screened strains that produce microbial oil by using straw as the substrate. Twenty-six isolates which had bigger and more oil bodies in their hyphae belong to five genera including *Microsphaeropsis, Phomopsis*, *Cephalosporium*, *Sclerocystis* and *Nigrospora*. Their oil contents ranged from 21.3 to 35.0% of dry cell weights when cultured in potato dextrose broth. When cultured on the solid- state medium composed of steam-exploded wheat straw (20% w/w), wheat bran (5%) and water (75%) they were able to produce cellulase and microbial oil with yields of 0.31 similar to 0.69 filter paper unit and 19 similar to 42 mg/g initial dry substrate, respectively. Peng et al. (2008) use *Microsphaeropsis* sp. to produce single cell oil (SCO) in solid-state fermentation from a substrate consisting of steam-exploded wheat straw and wheat bran. To achieve a higher yield, cellulase was added to the solid-state medium, resulting in an increase of SCO from 42 to 74 mg/gds with cellulase loading 10 FPU/gds. Fed- batch culture was carried out to produce intracellular lipid by limiting the nitrogen source for the cells previously grown under oxygen limiting condition. Number of cells remained constant after the exhaustion of nitrogen source and content of the intracellular lipid increased up to 40% (Pan et al. 1986). An endophytic fungus was screened from the phloem of *Populus euramevicana*, which can utilize cellulose to produce lipid (Jiang et al., 2009). It was identified as *Phoma* sp. On the 8th day, the highest lipid content and filter paper activity were 0.78 g/l and 5.67 mg/(L·min) (using glucose as substrate) respectively. The optimum condition for producing microbial oil included: corn stalks 50 g/l, NH_4NO_3 3 g/l, 25°C, 150 r/min. Lipid productivity thus reached 1.12 g/l. The oils produced under this condition were studied by GC, the main components were palmitic acid, stearic acid, oleic acid and linoleic acid, which could be used as raw materials of biodiesel.

Huang et al. (2009) use rice straw pre-treated with sulphuric acid and then detoxification including over liming, concentration and adsorption by amberlite XAD- 4 to produce microbial oils by *Trichosporon fermentans*. A total biomass of 28.6 g/l with a lipid content of 40.1% (corresponding to a lipid yield of 11.5 g/l) could be achieved after cultivation of *T. fermentans* on the detoxified SARSH for 8 days. Gao et al. (2009) found the optimum conditions for *Cunninghamella echinulata* B5 s unsterilized solid-state fermentation to produce lipid were: used 5g corn straw as basic solid culture medium, added 3.50 g glucose and 0.15 g ammonium nitrate and then added 0.025 mg citric acid, 0.02 g zinc sulfate and 8 ml water, inoculated with 10 mL cultivated seed liquid. On the basis of these conditions, along with natural pH, 25°C and 8d s fermentation, the percentage of lipid content could remain about 13% in dry substrate. Peng et al. (2009) use bagasse hydrolyzate detoxified with 3% activated charcoal (w/w) at a temperature of 80 and at a pH value of 2.0 for 50 min, more than 90% of the lignin decomposition products were removed. Being neutralized by $Ca(OH)_2$ and then the detoxified hydrolyzate was further used as carbon source for yeast lipids fermentation under nitrogen-limited conditions by *R. toruloides* AS2.1389. And the lipids content reached to 32.99% w/w based on dry cell weight, with lipid yield of 12.48 g lipid/100 g sugar. Dai et al. (2007) screened eight oleaginous yeasts among 250 yeast strains for xylose assimilating capacity. One strain (T216, *R. glutinis*) was found to produce lipids up to 36.6%. Under optimal fermentation conditions, *R. glutinis* accumulated lipids up to 49.25% on a cellular biomass basis and the corresponding lipid productivity reached 14.66 g/l. Experiments with a 5-L bioreactor under the optimal culture conditions showed that *R. glutinis* accumulated lipids up to 60.69%, resulting in 23.41 g/l in lipid productivity. More encouraging results were observed for the lipid production with alternative carbon sources. Corn stalk and *Populus euramevicana* leaves hydrolyzate could be used to substitute glucose. Chemical analysis indicated that biodiesel obtained by trans-esterification possessed similar composition to that from vegetable oil.

Xu et al studied heterotrophic growth of *Chlorella protothecoides* and obtained cell concentration 15.5 g/L and crude lipid content of 55.2% (Li et al., 2007; Wu et al., 2006; Xu et al., 2006; Hassan et al., 1993) optimized the growth of *Apiotrichum curvatum* in a continuous culture system using glucose and reached a lipid production rate of 0.42 g/lh and lipid content of 31.9% (w/w); Gouveia et al. (2009) used *Neochloris oleabundans* UTEX 1185 as a renewable lipid source for biofuel production and obtained an average lipid volumetric productivity of 37.66 mg /lday . Li et al. (2007) used pilot-scale fed batch cultures in a 15 L stirred tank fermenter for 134 h and resulted in dry biomass, lipid content and lipid productivity of 106.5 g/l, 67.5%(w/w) and 0.54 g/lh, respectively.

The diverse oleaginous microorganisms could accumulate lipids and metabolize different carbohydrates, which originate from renewable lignocellulosic biomass. Further research should be based on biochemical engineering technology and molecular biology which enhance the ability to assimilate different carbohydrates and accumulate high content of lipids. For example, Kalscheuer et al. (2006b) developed a microbial process to produce biodiesel-adequate fatty acid ethyl esters (FAEEs). Heterologous expression of two genes which encoding acyltransferase, pyruvate decarboxylase and alcohol dehydrogenase in the recombinant host *E. coli*, resulted in significant FAEE biosynthesis. Courchesne et al. (2009) demonstrated that we can enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches. Thus, it would be hopeful that the microbial oil be available for biodiesel production in the near future (Hahn-hagerdal et al., 2007; Zhao, 2005a).

On the other hand, many microbes are known to produce various alkanes of chain length ranging from C_{16} to C_{35} and commonly make long-chain hydrocarbons along with a series of low-molecular mass alcohols, ethers, esters and so on. Microbes producing such high-energy substances are of extreme general interest given the world's general need for alternative fuel sources (Strobel et al., 2008). There is currently substantial interest in utilizing eukaryotic algae for the renewable production of several bio-energy carriers, including starches for alcohols, lipids for diesel fuel surrogates and H_2 for fuel cells and genetic techniques can be develop to genetically optimize the production of targeted biofuels (Beer et al., 2009).

The current status and path to commercialization of microdiesel from biowastes

Currently, biofuels including microdiesel are much expensive than petroleum fuels and several problems must be solved for the commercialization of microdiesel from biowastes. Firstly, the acid used in the hydrolysis process would impose severe problem to the environment and the enzymes used in the process are rather expensive compared acid hydrolysis. In order to solve the problem, a reduction in cellulase production cost, an improvement in cellulase performance and an increase in sugar yields are all vital to reduce the processing costs; thermostable enzymes also offer potential benefits in the hydrolysis process (Gray et al., 2006; Ropars et al., 1992; Viikari et al., 2007; Zhang et al., 2006) . In the long run, enzyme production may take place in the same reactor as the hydrolysis and fermentation, which may ultimately be most efficient and most economic (Hamelinck et al., 2005; Merino et al., 2007).

Exogenous depolymerization enzymes used in the hydrolysis process may be replaced with plants that are capable of synthesizing these enzymes *in situ* and their cellulase and hemicellulase produced can break down cell walls just before harvest. This would greatly reduce the cost of raw material pre-treatment (Arthur et al., 2006; Sticklen et al., 2004; Sticklen, 2006). Secondly, as the accumulation of microbial oil consumes oxygen and needs a lot of energy, effort to reduce energy consumption is a key towards the industrialization in comparison with ethanol production. As the bioreactors need lots of energy to operate, which means a great amount of energy is consumed in the fermentation process. More efficient and energy saving bioreactors must be designed for the commercialization of microdiesel. Thirdly, fed-batch and continuous fermentations should be used to obtain high density cultivation of oleaginous microbes which would reduce the costs of microbial oil production (Li et al., 2007).

Of course, extracting the value added chemicals from biomass and microbes is a very important step in reducing the microdiesel production cost (Buzzini et al., 1999; Kamm et al., 2007) and the best driver is a clear policy environment that encourages the use of biomass energy such as purchasing incentives, tax credits or mandates (Wassell et al., 2006). The integrated biorefinery is an approach inevitable which optimizes the use of biomass for the production of microdiesel for both short and long term sustainability. There is just a need to allocate necessary resources for improving these technologies and plan its wide dissemination (Mirza et al., 2007).

CONCLUSION

The global demand for food is expected to double within the coming 50 years and the global demand for transportation fuels is expected to increase even more rapidly, the petroleum prices are likely to be much higher in the near future and it would be impossible to produce biofuels from food based crops. Thus, it would be inevitable to develop biofuels from biowastes. With the development of biotechnology and bioengineering, microdiesel will be economically competitive with petroleum fuel in the near future.

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